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By WILLIAM ALPHONSO MURRILL

A DISSERTATION SUBMITTED IN
PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
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ANALYSES OF URINARY PROTEIN AND VARIOUS FRACTIONS OF HUMAN AND PIG SERUM PROTEIN

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Interest in the chemical composition and identification of urinary protein has been stimulated by the work of Block *et al.* (1) who suggest that urinary protein is chemically identical with total serum protein and is changed to an albumin in the urine by the physical environment. For the proof of such a concept it is essential to have exact and complete amino acid analyses of urinary and serum proteins. One of the purposes of this paper is to furnish such analyses.

Further interest is aroused by Block's (2) separation of serum protein by various salting-out procedures into many protein fractions with widely different molecular ratios of the amino acids, arginine to lysine (10:9 to 10:92). This variation in ratio suggests the possibility of the formation of artificial products from one large aggregate by the reagents employed rather than a separation of the serum proteins into known entities of albumins and globulins.

EXPERIMENTAL

The serum was separated from 1500 ml. of blood obtained from three healthy young men and combined for analysis. 200 ml. of serum were coagulated by heat at pH 5, filtered, and the precipitate washed with hot water until chloride- and sulfate-free.

* The data in this paper have been taken from a thesis presented by William A. Murrill to the Horace H. Rackham School of Graduate Studies of the University of Michigan in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

It was then washed several times with 95 per cent alcohol, once with absolute alcohol, and several times with ether and dried in a vacuum desiccator over sulfuric acid for several days. This was considered to be total normal serum protein. The remaining 430 ml. of serum were fractionated quantitatively into albumin and globulin by the procedure of Howe (3) and washed and dried as before. In addition, a 500 ml. sample of blood was obtained from a nephritic patient who had a daily output of 7 gm. of urinary protein. The serum was separated and total serum protein was prepared as above.

Urinary protein (Sample A) was collected from this same patient over a period of several weeks, while subsisting on a diet free of egg protein. The intake was 100 gm. of protein and 3000 calories per day. A second sample of urinary protein (Sample B) was obtained over a period of 2 weeks while this individual was fed a diet in which the protein (35 gm.) was supplied essentially by eggs and which yielded 1500 calories per day. In both cases the collection of urinary protein was started after the subject had been on the diet for 1 week.

The albumin to globulin ratios of the serum and urinary proteins were obtained by the method of Howe (3). Moisture and ash were determined by the usual procedure for the urinary protein samples. The values for serum protein were corrected for ash and moisture by the following procedure. Assuming 16 per cent as the average nitrogen content for serum proteins corrected for moisture and ash, we multiplied our various analytical values by the ratio of 16 over the experimentally determined nitrogen content. The total nitrogen was obtained by the micro-Kjeldahl method of Pregl and amide nitrogen by the aeration method (4). Total sulfur was determined by the Parr bomb method. Cystine was determined by the Sullivan method, as modified by Rossouw and Wilken-Jorden (5), tyrosine and tryptophane by the methods of Folin and Marenzi (6), and basic amino acids by the methods of Block (7). The histidine was isolated by use of nitranilic acid (7).

DISCUSSION

The total nitrogen content of the various fractions of normal serum protein varied from 13.48 to 14.72 per cent and was in

general of the same order of magnitude as that of nephritic total serum protein (Table I). The variation probably was due to a difference in the moisture content, as shown in the case of the two samples of urinary protein, the values being 16.26 and 16.16 per cent respectively when corrected for ash (Sample A, 0.23 per cent; Sample B, 0.09 per cent) and moisture (Sample A, 8.8 per cent, and Sample B, 4.9 per cent). A significant difference was found in the amount of cystine, tyrosine, tryptophane, lysine, and ar-

TABLE I

Analyses of Human Serum and Urinary Proteins

All values except the total nitrogen are corrected for ash and moisture as described in the text.

Protein	Albumin Globulin	Total N	Sulfur	Cystine	Sulfur accounted for by cystine	Tyrosine	Tryptophane	Histidine	Arginine	Lysine
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Total serum, normal....	2.0	14.28	1.62	4.32	71	5.56	1.65	2.47	5.20	9.63
“ “ nephritic....	2.6	14.72	1.62	4.24	70	5.45	1.58	2.53	5.22	9.64
Albumin, normal.....		13.48		4.75		4.94	0.82	2.58	5.30	10.40
Globulin, “		14.00		3.43		6.38	2.42	2.34	4.70	7.00
Theoretical values*.....				4.70		5.0	0.88	2.57	5.28	10.27
Urine protein, Sample A†	24	14.80	1.84	5.7	82.4	5.1	0.90	2.46	5.70	10.46
“ “ “ B.	24	15.33	1.87	6.0	86.1	5.2	0.92	2.58	5.50	10.35

* Values calculated for a serum protein with an albumin to globulin ratio of 24:1, our values for albumin and globulin being used. See the text.

† Samples A and B were collected while the subject was on different diets. See the text for the details.

ginine in serum globulin as compared with serum albumin (Table I).

The values for sulfur and various amino acids in total normal serum protein are in close agreement with those obtained for total nephritic serum protein (Table I). These findings are in agreement with those of Metzger (8) and Tuchman and Reiner (9). However, in view of the work of Lang and Braun (10), Schenck and Kunstmann (11), Alving and Mirsky (12), and Dirr (13)

we might have expected differences, because these workers present evidence to show that serum proteins are not of constant composition.

The percentages of sulfur and of all the amino acids determined except histidine are significantly different in albumin and globulin. In our preparation the cystine content of human serum albumin was 4.75 per cent as compared to 3.43 per cent for the globulin. Other workers (8, 14-17) find similar differences in the cystine content of serum albumin and globulin obtained from different species (human, horse, goose, and ox). Hewitt (18) separated serum albumin into three fractions and obtained 5.8 per cent cystine as the highest value, in the fraction referred to as crystalalbumin. The lowest cystine content of an albumin fraction (1.8 per cent) was found in seroglycoid. Abderhalden and Siebel (19) obtained for horse serum albumin 4.5 per cent tyrosine and 0.83 per cent tryptophane, and for globulin 5.73 per cent tyrosine and 2.12 per cent tryptophane. These are in agreement with our values for human serum albumin (tyrosine, 4.94 per cent; tryptophane, 0.82) and globulin (tyrosine, 6.38 per cent; tryptophane, 2.42). The histidine values obtained by us are higher than those previously reported (1, 20) for serum proteins. This can be explained by the fact that we used the nitranilic acid method of Block (7), which gives higher values. No differences between the histidine content of human serum albumin and human serum globulin were observed. This is contrary to the findings of Block (20) who made similar studies with cattle serum. The arginine and lysine values for albumin are 5.3 and 10.4 per cent respectively as contrasted to 4.7 and 7.0 per cent for the globulin. These absolute values are higher but the relative values are similar to those for cattle serum (20).

In order to ascertain whether urinary protein is total serum protein (1, 21) or mostly albumin (22), comparison of the sulfur and amino acid contents of the urinary protein, total serum protein, albumin, and globulin is significant (Table I). The cystine values for urinary protein (5.7 and 6.0 per cent) are higher than those for our unpurified sample of serum albumin but agree with the value of 5.8 per cent for crystalalbumin (23) and with the figure of 6.07 per cent obtained for human serum albumin by Tuchman and Reiner (9). If the values obtained for tyrosine,

tryptophane, arginine, and lysine of human serum albumin are compared with the urinary protein values (Table I), one observes very close agreement. The albumin to globulin ratio for urinary protein was found to be 24:1 (Table I). A close agreement exists between the amino acid composition of the urinary protein as determined and that which would be anticipated if the urinary protein were a mixture of serum proteins in the above ratio (designated as "Theoretical values" in Table I) or entirely serum albumin. The evidence here presented suggests that serum protein is composed of at least two distinctly different protein com-

TABLE II
Analyses of Pig Serum Protein Fractions

All values except the total nitrogen are corrected for ash and moisture as described in the text.

Fraction	Nitrogen			Cys- tine	Tyro- sine	Tryp- to- phane	His- tidine	Argi- nine	Lysine
	Total	Amide of total	Hu- min of total						
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Saturated MgSO ₄ fil- trate, 25°	14.01	6.81	1.76	5.39	5.20	1.08	2.9	5.8	9.5
30% Na ₂ SO ₄ ppt.	14.35	8.35	3.00	2.84	5.92	1.23	*	5.7	8.4
20% MgSO ₄ "	13.35	9.46	1.60	3.08	6.04	2.13	2.0	5.3	6.9
Saturated MgSO ₄ ppt., 37°	15.22	8.20	1.83	2.75	5.90	2.16	2.0	5.4	6.5
15% Na ₂ SO ₄ ppt.	15.08	8.03	1.60	2.76	6.10	2.13	1.8	5.3	6.2

* Determination lost.

ponents, since the albumin in the native state must have different properties from the other constituents to be selectively excreted through the kidney.

Serological comparisons also indicate to us that urinary protein is not total serum protein but a fraction thereof.¹

Our findings that urinary protein is not identical with total serum protein in so far as amino acid content is concerned does not corroborate the work of Block *et al.* (1) who found that urinary protein is the same as total serum protein. In view of this dis-

¹ Murrill, W. A., Soule, M. H., and Newburgh, L. H., unpublished data.

crepancy and since Block used this finding as evidence that serum protein fractions as ordinarily prepared are artifacts rather than real entities, we fractionated fresh pig serum by the salting-out procedures of Block (2) and determined on each fraction nitrogen, sulfur, and six amino acids (Table II). These data indicate no great variation in the amino acid values. The differences are of the same order of magnitude that exist between the contents of the amino acids found for human serum albumin and globulin (Table I). The greatest variations of fractions of pig serum were in the cystine (2.75 to 5.39 per cent), tryptophane (1.08 to 2.16 per cent), and lysine (6.2 to 9.5 per cent) contents. The arginine to lysine ratios varied from 10:14 to 10:20 as contrasted to 10:9 to 10:92 reported by Block for cattle serum (2). The data afford no evidence that the experimental procedure of salting-out effects any significant fractionation other than the usual separation into the components commonly designated as albumin and globulin.

SUMMARY

1. A comparative study of the composition of serum albumin, serum globulin, and urinary protein has been made by determining the sulfur, nitrogen, and various amino acids. The results indicate that urinary protein is either all serum albumin or is a mixture correctly represented by the albumin to globulin ratio as found in the urine.

2. Two samples of urinary protein collected while the subject subsisted on different dietary régimes show identical analyses.

3. Pig serum was fractionated by the procedures of Block and the various fractions were analyzed. No evidence was obtained to indicate that the various fractions differed widely in composition, notably in their lysine content, as reported by Block for the protein fractions of beef serum.

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